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NARRATIVE REVIEW

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The relevance of intestinal barrier dysfunction, antimicrobial proteins and bacterial endotoxin in metabolic dysfunction-associated steatotic liver disease

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Abstract

Background: Metabolic dysfunction-associated steatotic liver disease (MASLD) is a leading cause of end-stage liver disease associated with increased mortality and cardiovascular disease. Obesity and diabetes are the most important risk factors of MASLD. It is well-established that obesity-associated insulin resistance leads to a situation of tissue lipotoxicity characterized by an accumulation of excess fat in non-fat tissues such as the liver, promoting the development of MASLD, and its progression into metabolic dysfunction-associated steatohepatitis.

Methods: Here, we aimed to review the impact of disrupted intestinal permeability, antimicrobial proteins and bacterial endotoxin in the development and progression of MASLD.

Results and Conclusion: Recent studies demonstrated that obesity- and obesogenic diets-associated alterations of intestinal microbiota along with the disruption of intestinal barrier integrity, the alteration in antimicrobial proteins and, in consequence, an enhanced translocation of bacterial endotoxin into bloodstream might contribute to this pathological process through to impacting liver metabolism and inflammation.

KEYWORDS

antimicrobial proteins, bacterial endotoxin, intestinal barrier, liver steatosis, obesity, obesogenic diet

1 | INTRODUCTION

Recently, a new consensus has been established on the terminology and diagnostic criteria for metabolic-associated fatty liver disease, which before some authors and institutions perceived as stigmatizing and caused some confusion due to changes in diagnostic criteria.[1] The new agreed terminology is metabolic dysfunction-associated

steatotic liver disease (MASLD). The diagnostic criteria of MASLD are defined as hepatic steatosis plus either type 2 diabetes, overweight/obesity, or two of dyslipemia, hypertension and prediabetes. In fact, obesity, insulin resistance and diabetes are the most important risk factors of MASLD that by now is a leading cause of end-stage liver disease associated with increased mortality and cardiovascular disease.[1–3]

MASLD involves a continuum of different liver conditions, ranging from simple fatty liver (hepatic steatosis), which can be detected through imaging or histological methods, to metabolic dysfunction-associated steatohepatitis (MASH), characterized by inflammation and more severe liver damage. It is well-established that obesity-associated insulin resistance leads to a situation of tissue glucotoxicity and lipotoxicity that results in excess fat accumulation in non-fat tissues, such as the liver, and, in consequence, promot the development of MASLD, and its progression to MASH.[4] Recent evidence demonstrated that obesity-associated changes of intestinal microbiota composition might contribute to this pathological process along with a disruption of intestinal barrier integrity, alterations in antimicrobial proteins and, in consequence, an enhanced translocation of bacterial endotoxin into bloodstream all impacting liver metabolism and inflammation.

The current narrative review focuses on checking the relevance of intestinal barrier integrity, antimicrobial proteins (focusing on LPS-sensitive proteins such as lipopolysaccharide binding protein, lipocalin 2, defensins and lactoferrin) and bacterial endotoxin in the development of MASLD and its progression into MASH.

2 | INTESTINAL BARRIER: STRUCTURE IN HEALTH

The intestinal barrier consists of a complex structure of several interacting layers, which besides being a gatekeeper for nutrient digestion and absorption also build a physical barrier for the entry of pathogens and so-called pathogen associated molecular patterns (PAMPs) from intestinal lumen to circulation. Results of studies suggest that an alteration of intestinal barrier function including even minor changes in the regulation of the interplay of epithelial, microbial, biochemical, or immunological barriers might contribute to the development of metabolic diseases including MASLD (for overview see Tilg et al.[5,6]). In the following, some of these complex structures discussed to be critical in the development of MASLD are briefly described with a specific focus on intestinal barrier and antimicrobial peptides as the role of alterations of intestinal microbiota has been discussed recently in great detail by others (for overview see Effenberger et al.[7] and Fujiki and Schnabl.[8]).

2.1 | Intestinal epithelial layer

Being layered on top of the intestinal epithelium, the intestinal mucus layer differing in composition and properties in different parts of the gastrointestinal tract, plays an important role as physical barrier. It has also been proposed to modulate the immune system and therefore is often considered as a 'first line defence' against external injuries (for overview also see Vancamelbeke and Vermeire[9], and Di Sabatino et al.[10]). In the small intestine, mucus typically is 'non-attached' to the epithelial cells and covers the villi tips. Based on findings in patients with cystic fibrosis, mucus function in this part of the gastrointestinal tract has been related not only to antimicrobial properties but also to cellular ion channel function.[10] In the large intestine, the mucus is organized as a double layer with the outer layer (also referred to as stirred mucus layer) being composed of mucins (mainly MUC2), soluble immunoglobulin A (IgA) and antimicrobial peptides. The inner and denser layer (also referred to as non-stirred mucus layer) being composed of net-forming MUC2 but also enterocyte surface glycocalyx composed of transmembrane mucins for example, MUC3, 12, 17 is strongly attached to the epithelia and is considered impermeable for microorganisms.[10]

The intestinal epithelium consists in both small and large intestine of a monolayer of absorptive enterocytes that are interspersed with multiple different cells such as enteroendocrine cells, goblet cells, Paneth cells, and microfold cells (M cells) (also see Figure 1) all differentiating from pluripotent intestinal stem cells located in the crypts.[10,12] At homeostasis, epithelial cells in the intestine are estimated to have a turnover of 4–7 days.[13] At the luminal, apical side intestinal epithelial cells are tightly connected through junctional complexes comprised of tight junctions while towards the basolateral side they are connected through adherence junction and desmosomes (for overview see[14]). In both the small and large intestines, tight junction proteins showing both size- and charge-selectivity are thought to be key components in the control of paracellular transport of the resulting semipermeable barrier (also see Odenwald and Turner[13]). It has been proposed that there are two distinct routes across tight junctions of an intact epithelial monolayer, the so called 'pore' and 'leak' pathways.[13] Herein, the pore pathway in which permeability seems primarily to be dependent upon the subset of claudins[15] whereas the 'leak' pathway has been proposed to be highly dependent upon ZO-1, occludin and myosin light

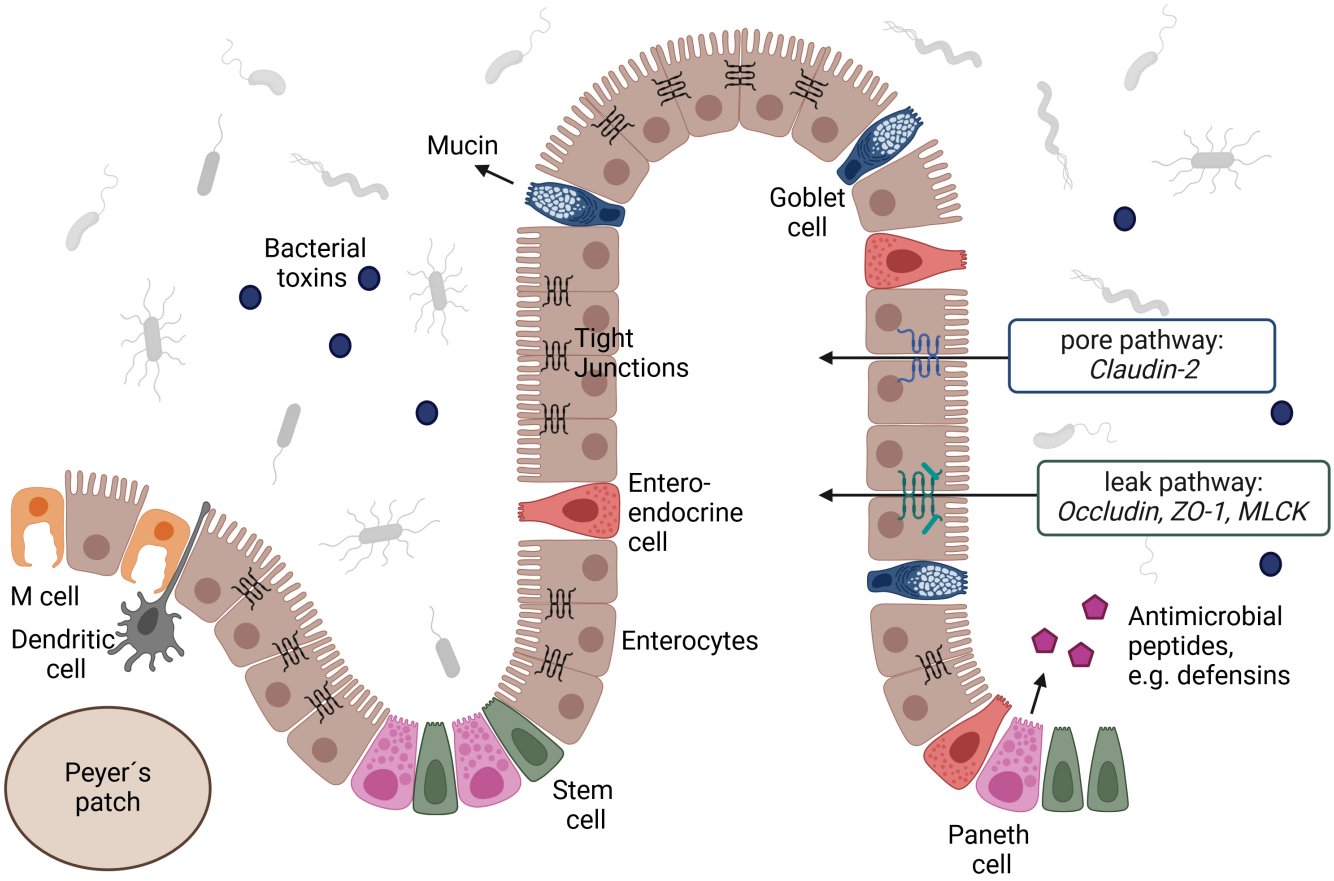

FIGURE 1 Schematic overview of cellular and molecular components of the intestinal barrier. Adapted from Untersmayr et al.[11] For further explanation also see main text. ZO-1, zonula occludens-1. Created with BioRender.com.

chain kinase (MLCK).[15,16] Indeed, studies have shown that MLCK activity modulated paracellular permeability through restructuring perijunctional F-actin and subsequently occludin and ZO-1.[17] Also, studies further suggest that posttranslational modifications like changes in phosphorylation of occludin and ZO-1 may modify intestinal barrier function.[11] Besides the enterocytes and goblet cells the latter being the main source of the mucins found in the mucus layer, Paneth cells are also critical in maintaining intestinal homeostasis and barrier function especially through secreting antimicrobial peptides.[10] Paneth cells-produced antimicrobial peptides, such as defensins, exhibit an important role in the maintenance of gut microbiota amount and composition and prevent intestinal bacterial overgrowth and gut dysbiosis, as described below.

Furthermore, it has been shown that bacteria-specific IgA being secreted by B cells upon an activation of dendritic cells which in turn have been shown to be activated by M cells also support the mucosal barrier by decreasing the penetration of bacteria (for overview also see Untersmayr et al.[11] and Kobayashi et al.[18]). However, M cells have also been suggested to function as special gateways for luminal antigen transport across the gut epithelium.[19]

2.2 | Epithelial-vascular barrier in the intestine

In recent years studies suggest that the gut-vascular barrier located beneath the intestinal epithelium forming the innermost layer of the intestinal wall defence system, may also be critical in the development of intestinal barrier dysfunction (for overview see Di Sabatino et al.[10]). The gut-vascular barrier is made up of a monolayer of endothelial cells which are sealed together by adherent and tight junctions being surrounded by pericytes and enteric glia cells. As the endothelial lining is fenestrated, the gut-vascular barrier represents a semipermeable structure allowing the diffusion of nutrients and luminal contents (up to a molecular weight of ~4 kDa).[10] Fluorescent molecules (such as fluorescein isothiocyanate (FITC)-dextran 4 kDa or Lucifer yellow) applied by gavage have been largely used to evaluate gut leakiness in diet-induced obesity and NAFLD (MASLD) mice experimental models.[20,21] Of note, results of a recent study suggest that in obesity experiments, the FITC-dextran dose should be adjusted based on lean body mass rather than body weight to avoid overestimating the degree of intestinal permeability in obese mice.[22] Studies also suggest that through

yet not fully understood mechanisms intestinal microbiota may modulate the epithelial-vascular barrier in the gut.[23] Specifically, studies suggest that certain pathogenic bacteria like *Salmonella typhimurium* may penetrate the epithelial-vascular barrier and that this is related to alterations of the β-catenin-dependent signalling in gut endothelial cells.[24]

3 | PATHOGEN-ASSOCIATED MOLECULAR PATTERNS AND PATTERN RECOGNITION RECEPTORS

The concept of pattern recognition receptors (PRR) recognizing pathogen-associated molecular patterns (PAMPs) and subsequently activating both innate and adaptive immunity was already described in 1989.[25] PRRs consist of a large variety of receptors including toll-like receptors (TLRs), nucleotide-binding oligomerization domain-like receptors, C-type lectin receptors and retinoic acid-inducible gene I-like receptors.[26] In the following a brief overview on TLRs and herein especially TLR4 is given as results of studies with rodents but also humans suggesting that the activation of TLRs and especially of TLR4 is critical in the onset and progression of MASLD[27,28] and alcohol associated liver disease (for overview also see Hartmann et al.[29] and Petrasek et al.[30]).

So far 10 TLRs have been described in humans that are expressed in innate immune cells like monocytes, macrophages, and dendritic cells but also in non-immune cells like epithelial cells and fibroblasts which can be distinguished in cell surface TLRs (e.g. TLR1, TLR2, TLR4, TLR5 and TLR6 as well as TLR10) and intracellular TLRs (e.g. TLR3, TLR7, TLR8 and TLR9).[31,32] Upon their cellular localization the TLRs located at the cell surface have been shown to predominantly recognize components of microbial membranes like lipids, lipoproteins, and protein.[31,33] TLR4 forming a complex with myeloid differentiation factor 2 (MD2) has been shown to recognize lipopolysaccharides (LPS) found in the outer-wall of Gram-negative bacteria (for overview see[34]), which is delivered to this TLR by CD14.[35] As reviewed in detail in other parts of this review, it has been suggested that the so called lipopolysaccharide binding protein (LBP), may also be involved in the delivery of LPS to the TLR4/MD2 complex[36]; however, data on the role of LBP in LPS-dependent signalling is still contradictory.[37] Once the TLR4/MD-2 heterodimer is activated, the intracellular signalling can follow two directions, the TLR4/MyD88/NFκB or the TLR4/TRIF/IRF3 pathway (see Figure 2 and[38] for an in-depth overview). Studies suggest that these two pathways are competitive[39] and that the TLR4/MyD88/NFκB pathway starts from the complex located on the plasma membrane while the TLR4/TRIF/IRF3 signalling cascades begins after the complex is internalized into endosomes.[38]

4 | INTESTINAL BARRIER DYSFUNCTION AND BACTERIAL ENDOTOXIN IN THE DEVELOPMENT OF MASLD

Throughout the last decades, not only alterations of intestinal microbiota composition but also changes of intestinal barrier function and subsequently an increased permeation of PAMPs have been associated with a variety of intestinal and systemic diseases including MASLD. And while the vast majority of these associations are merely correlative, by now some experimental evidence relating intestinal barrier dysfunction and elevated PAMP levels, and herein especially bacterial endotoxin to disease pathogenesis exist for some diseases including MASLD. Indeed, in both children and adults with different stages of MASLD/ MASH, it has been shown that bacterial endotoxin levels are higher than in healthy controls, and that this is related to increased markers of intestinal permeability and a loss of tight junctional proteins in the duodenum.[40–47] Recently, results of a meta-analysis even suggested that blood endotoxin levels may be suitable as biomarker of MASLD.[48] Also, results of studies in animals suggest that these alterations may even occur in the absence of overweight or obesity and seem also to be related to diet (e.g., the intake of specific macronutrients like fructose).[49–51] Further supporting the hypothesis that an elevated permeation of bacterial endotoxin may be critical in the development of MASLD, it has been shown that MASLD patients also frequently show elevated plasma levels of the CD14, LBP as well as enhanced expression of the endotoxin receptor TLR4 and tumour necrosis factor (TNF)-α in liver tissue.[52–54] Interestingly, in livers of patients with simple steatosis but even more so in those with MASH and MASH with beginning fibrosis expression of other TLRs for example, TLR1-5 (but not TLR6-10) was also found to be also induced.[27] This further suggests that a permeation of other PAMPs may also be altered in MASLD patients. The hypothesis that an increased permeation of bacterial endotoxins from the small and large intestine and subsequently an activation of TLR4 dependent signalling cascades in the liver may contribute to the development of MASLD and especially MASH has also been supported by numerous studies in model organisms like ob/ob and db/db mice, or when MASLD was induced by different diets (e.g. diets rich in fat, fructose and/ or cholesterol).[20,28,55–62] Furthermore, targeting intestinal microbiota for example, through treating animals with

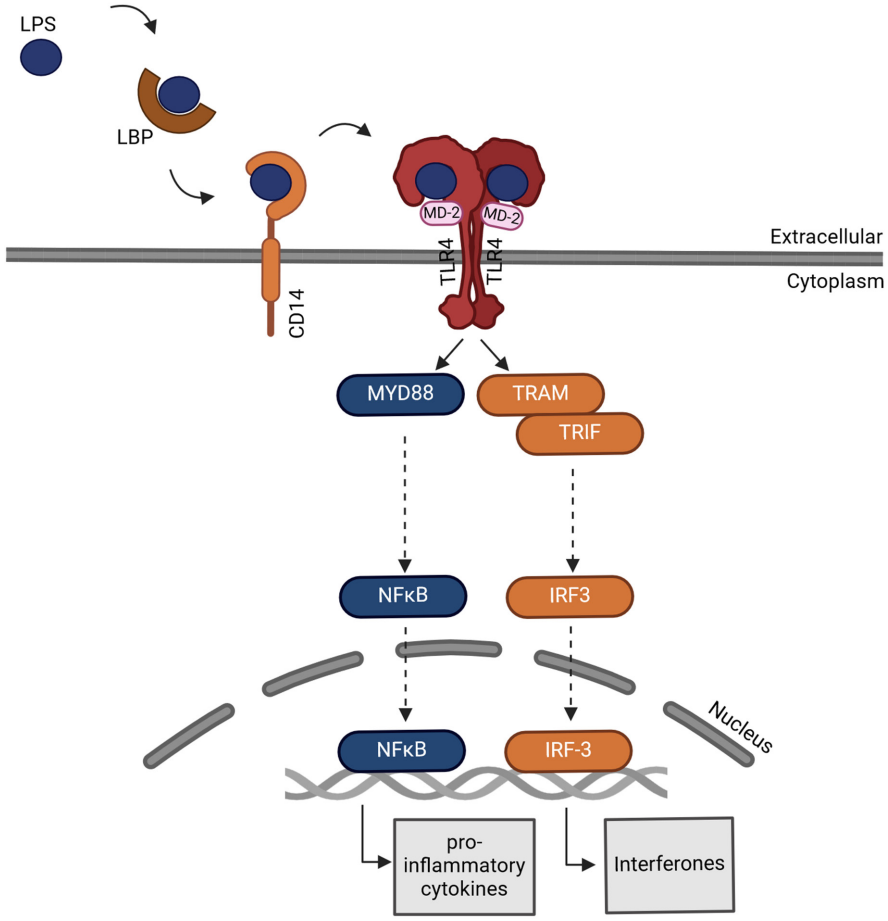

FIGURE 2 The TLR4 signalling cascade. TLR4 is bound to the cell membrane and activated by the recognition of lipopolysaccharides (LPS, endotoxin). Once activated proinflammatory cytokines are released through the MyD88-dependend activation of NFκB or interferon regulatory factor 3 (IRF3)-dependent signalling cascades. CD14, cluster of differentiation 14; LBP, lipopolysaccharide binding protein; MYD88, Myeloid differentiation primary response 88; NFκB, Nuclear factor kappa-light-chain-enhancer of activated B cells; TLR4, toll-like receptor 4; TRAM, TRIF-related adaptor molecule; TRIF, TIR domain-containing adaptor protein. Created with BioRender.com. Adapted from.[118]

a mix of antibiotics targeting Gram-positive and Gram-negative bacteria while feeding them a MASLD-inducing diet thereby reducing prevalence of bacteria >90%–95%, has repeatedly been shown to be related with a marked dampening of the development of MASLD.[49,63,64] This was also associated with a diminished TLR-response and activation of downstream signalling cascades in the liver.[61] However, results of studies employing (sub-) therapeutic doses of different antibiotics resulting in a decrease of microbial diversity and relative abundance have been shown to be related with an exacerbation of the diet-induced MASLD.[65] In line with these findings, studies in human and mice employing rifaximin may have beneficial effects on MASLD if bacterial composition is shifted.[66] Recently, it has been shown in mice concomitantly treated with an antibiotic mix while being fed a MASLD-inducing diet bacterial endotoxin levels and TLR4 mRNA expression in liver was at the level of controls. Interestingly, intestinal barrier function was similarly disturbed as in MASLD-diet fed mice not treated with the antibiotic. In this study it was further shown that intestinal barrier dysfunction (e.g. the loss of tight junctions and increased permeability) may be attributed to macronutrients for example, fructose, found in the MASLD diet and their metabolism in small intestinal tissue and subsequent alterations of intestinal NO-homeostasis.[63] Also, the development of MASLD has been shown to be markedly diminished in studies in rodents with genetic modifications and pharmacological interventions targeting NO-metabolism and the loss of tight junction proteins in intestinal tissue[51,67–69] or the activation of TLR4 and depending signalling cascades in the liver[28,51,67,70] (for overview see also Figure 3). In addition, some bacterial metabolites such as butyrate, improved gut barrier function acting on tight junctions[71,72] and attenuated fructose-induced hepatic lipid accumulation and inflammation, possibly, by enhancing duodenal melatonin synthesis.[62]

5 | THE RELEVANCE OF BACTERIAL ENDOTOXIN-SENSITIVE ANTIMICROBIAL PROTEINS ON MASLD PROGRESSION

Obesity, being a key risk factor for the development of MASLD leads to systemic immunologic alterations, which are associated with an unbalanced production and secretion of antimicrobial proteins from the first line of defence derived classically from circulating leukocytes, liver, fat, lungs, and intestines. Altered levels of

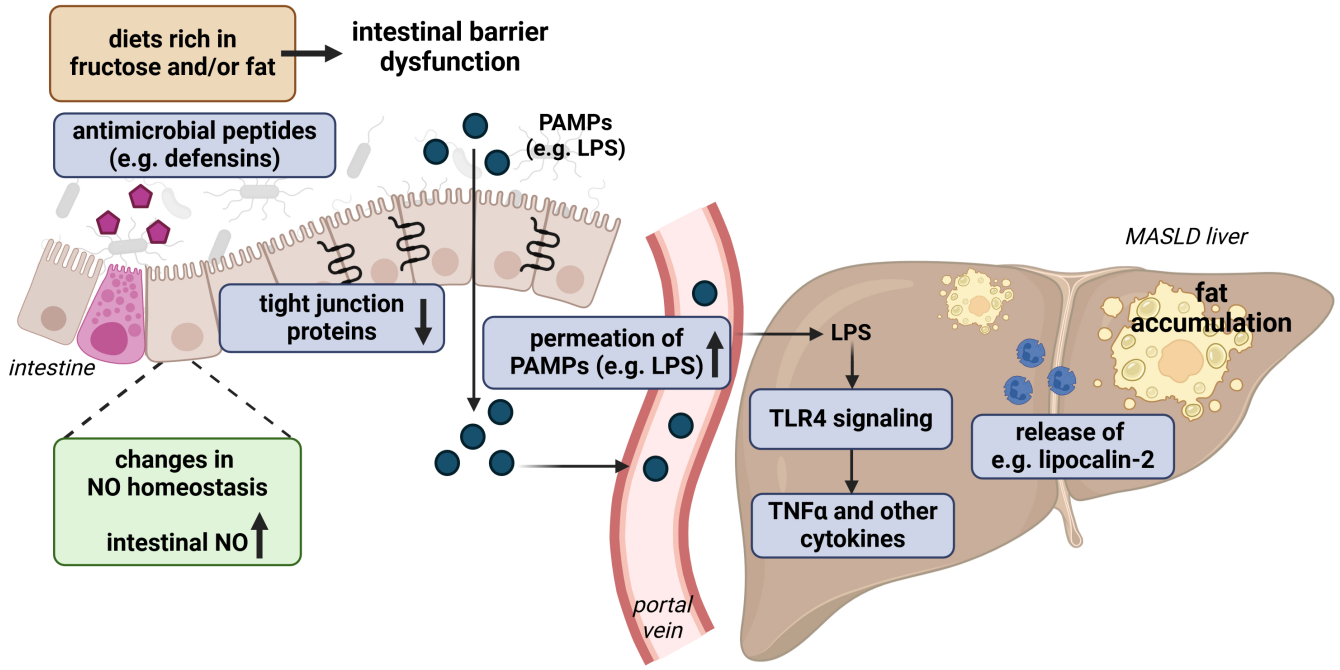

FIGURE 3 Intestinal barrier dysfunction, PAMPs and antimicrobial peptides in the development of MASLD. Alterations of bacterial composition and intestinal permeability as well as antimicrobial peptides related to the prevalence of obesity and intake of macronutrients like saturated fats and/ or fructose, gut derived PAMPs like LPS (bacterial endotoxin) cross the intestinal barrier and lead to an activation of TLR4-dependent signalling cascades and the release of pro-inflammatory cytokines like TNFα in the liver. LPS, lipopolysaccharide; MASLD, metabolic dysfunction-associated steatotic liver disease; NO, nitric oxide; PAMPs, pathogen-associated molecular patterns; TLR4, Toll-like receptor 4; TNF, tumour-necrosis factor alpha. Created with BioRender.com. Adapted from.[119]

circulating and tissue bacterial endotoxin-sensitive antimicrobial proteins have been proposed as a potential trigger of obesity-associated metabolic disturbances, such as insulin resistance, fat liver accumulation, adipose tissue dysfunction and gut dysbiosis.[73] In fact, a large number of evidences support a relevant role of some of these bacterial endotoxin-sensitive antimicrobial proteins in MASLD progression.

5.1 | Bacterial endotoxin-sensitive antimicrobial proteins promoting MASLD

5.1.1 | Lipopolysaccharide binding protein

LBP is an acute-phase protein produced mainly by hepatocytes, and secondly by adipocytes, that circulates in the bloodstream as a marker of endotoxemia and indirect biomarker of intestinal permeability.[41,74] LBP biosynthesis is enhanced in response to LPS and other proinflammatory stimuli leading to liver damage.[74] In fact, LBP binds to the lipid A portion of bacterial endotoxin and facilitates its interaction with TLR4/MD2/CD14 protein complex to activate pro-inflammatory pathways of innate immunity through the induction of NF-κB and activator protein 1, the major transcription factors involved in inflammation[75] (see Figure 2). In the last 15 years, increased circulating LBP levels have been strongly linked to obesity, insulin resistance and MASLD in children and adults.[41,76–80] Mice experiments indicated a possible role of LBP in MASLD progression, but only in mice fed with obesogenic diet. For instance, in mice fed with a high-fat and high-sucrose diet, *Lbp* gene deletion (LBP KO mice), or gene knockdown using small interference RNAs against *Lbp* mRNA carried in liver-targeted nanoparticles, resulted in a significant improvement of liver steatosis through the attenuation of diet-induced hepatic lipogenesis-, fibrosis- and inflammatory-related pathways.[70,81] In part, these beneficial effects on liver inflammation might be mediated by diminishing LPS signalling in liver.[70] However, the effects observed in relation to hepatic lipid metabolism (triglyceride accumulation and lipogenesis) did not appear to be dependent on the action of LPS, but could be due to a direct effect of LBP on lipid droplet development or lipogenesis induction.[81] As mentioned above, some ambiguity in the role of LBP in LPS-dependent signalling has been reported.[37] In fact, in absence of obesity or diet-induced hepatic lipogenesis, liver LBP seems to exert a protective role in the prevention of liver inflammation, oxidative stress and fibrosis, suggesting that the inhibition of LBP might magnify proinflammatory effects of LPS.[82] Although previous experimental studies in mice and rats supported

this idea,[83–86] experiments in hepatocytes demonstrated a proinflammatory effect of LBP gene knockdown in the absence of LPS, which could be produced by other causes, such as oxidative stress and perturbations in cellular lipidomic signature.[82,87] Furthermore, two recent studies go against the detrimental effect of LBP on MASLD progression in obesity,[88,89] suggesting that further mechanistic studies are required to clarify the impact of LBP on liver physiology and metabolism, and molecular processes underlying these effects.

5.1.2 | Lipocalin 2

Lipocalin 2 is a neutrophil gelatinase-related lipoprotein with antimicrobial activities expressed in liver under inflammatory conditions and in response to bacterial endotoxin.[90] Increased tissue and circulating lipocalin 2 levels have been largely shown in subjects with obesity in association with insulin resistance and MASLD.[91–93] A recent study demonstrated that lipocalin 2 promotes liver inflammation and fibrosis through the activation of hepatic stellate cells via α-SMA/matrix metalloproteinase 9 (MMP9)/signal transducer and activator of transcription 3 (STAT3) signalling. In consequence, MASH was aggravated in wild type and ob/ob mice fed with high-fat diet for 20 weeks.[94] Additionally, another recent study found that recombinant FGF21 diminished polychlorinated biphenyls-induced MASLD and MASH in high-fat diet-fed mice by attenuating hepatic lipocalin 2 expression,[95] supporting lipocalin 2 as a putative target to improve MASLD.

5.2 | Bacterial endotoxin-sensitive antimicrobial proteins attenuating MASLD

5.2.1 | Defensins

As mentioned above, obesity and the intake of specific macronutrients (e. g. saturate fat and fructose), are associated to perturbations in the intestinal mucosal barrier that might impact on liver steatosis. Defensins exert a relevant role in the intestinal barrier's first line of defence, and are widely produced in several tissues, for instance α-defensins 5 and 6 in Paneth cells in the intestine, β-defensins in epithelial surfaces, colon and liver.[96] Studies in humans and mice demonstrated a negative association between obesity and intestinal α-defensins mRNA levels.[97,98] Of note, decreased intestinal α-defensins expression led to gut dysbiosis and bacterial overgrowth, and disrupted intestinal mucosal integrity.[99,100] In mice, exogenous oral administration of human α-defensin 5 and β-defensin 2 or overexpression of ileal defensins resulted in a significant improvement diet-induced hepatic steatosis.[96,98,101,102] It has been suggest that an enhanced gut barrier function, which includes the induction of tight junction protein expression and small intestinal host defence peptides,[96] and an attenuation of the pro-inflammatory effects of bacterial endotoxin may be critical herein.[103] Additionally, administration of full-length human α-defensin 5 to high fat diet (HFD)-fed mice during 10 weeks displayed a positive role attenuating dyslipidemia and circulating free fatty acid levels,[102] and administration of human β-defensin 2 exerted hepatic protective effects, as reflected by improving alcohol-related liver disease and reducing plasma alanine transaminase activity in mice fed with an ethanol-containing diet.[104] In addition to intestinal defensins, the induction of systemic α-defensin, by its overexpression in polymorphonuclear leukocytes, also exhibit important benefits in the prevention of MASLD by improving lipid metabolism and attenuating liver fat accumulation.[105]

5.2.2 | Lactoferrin

Lactoferrin is a multi-functional and pleiotropic glycoprotein of the innate immune system that is produced by neutrophils and glandular epithelial cells. One of the most relevant functions of lactoferrin is its immunomodulatory capacity in response to several PAMPs (such as bacterial endotoxin), by attenuating the pro-inflammatory activities of these stimuli.[73]

Studies in humans reported that circulating lactoferrin levels were decreased in patients with obesity and type 2 diabetes, and negatively correlated with obesity-associated metabolic disturbances, including insulin resistance and dyslipidemia.[106,107] Ex vivo and in vitro experiments demonstrated that insulin resistance reduced lactoferrin biosynthesis,[107,108] and that exogenous lactoferrin administration impacts positively on insulin action and adipogenesis, in part due to the inhibition of pro-inflammatory pathways.[109,110] In line with these observations, in the last 10 years, some beneficial metabolic effects of lactoferrin like improving weight gain, insulin resistance, glucose tolerance, dyslipidemia and liver steatosis in obesogenic conditions, have been shown in several mouse studies.[111–116]

In 2014, Li et al reported that high-fructose diet promotes increased gut bacterial 'bloom', intestinal permeability and bacterial endotoxin translocation into blood and liver in association to hepatic lipid accumulation and inflammation. In this context, lactoferrin administration prevents the negative effects high-fructose diet on liver steatosis, possibly, by attenuating the bacterial

TABLE 1 Summary of the most relevant studies showing the importance of intestinal barrier dysfunction, endotoxin, LBP, lipocalin 2, defensins and lactoferrin on MASLD.

Study	Main findings	Based on
[45]	A pioneer study showing the relationship between NAFLD and intestinal permeability in humans	Clinical observations in humans
[49]	Short-term intake of diets that promotes hepatic steatosis, led to altered intestinal barrier function	Fat-, fructose- and cholesterol-rich diet mice experiments
[28]	LPS/TLR4 signalling mediated hepatic steatosis induced by chronic intake of high fructose solution	Diet and TLR4 mutant mice experiments
[41]	Increased plasma endotoxin levels were associated to early stages of NAFLD in children	Clinical observations in humans
[48]	Blood endotoxin levels could be used as a relevant diagnostic biomarker for NAFLD	A meta-analysis that includes 34 studies in humans
[77]	Increased endotoxin levels were associated to NASH and liver fibrosis	Clinical observations in humans
[70]	Compared to wild type, LBP KO mice fed with a high fat, fructose and cholesterol diet showed less liver damage, inflammation and steatosis	Diet and LBP KO mice experiments
[76]	Serum LBP levels could be a potential biomarker of liver fibrosis in NAFLD	Clinical observations in humans
[80]	Circulating LBP levels were associated to hepatic fat fraction and liver volume in adolescents with obesity	Clinical observations in humans
[81]	Liver Lbp depletion using chemically modified siRNAs resulted in reduced lipid accumulation, lipogenesis and lipid peroxidation	In vitro and mice experiments. Clinical observations in humans
[92]	Serum lipocalin 2 levels were significantly increased in NAFLD patients, and correlated with steatosis, inflammation, fibrosis score, and NAFLD activity score	Clinical observations in humans and mouse NASH model
[94]	Lipocalin 2 increased hepatic stellate cells activation via SMA/MMP9/STAT3 signalling enhancing NASH	High-fat diet and lcn2 KO mice experiments
[98]	Dysfunctional Paneth cells in obesity reduced α-defensin levels in the intestinal lumen in association with NASH progression. Oral administration of α-defensins in this situation attenuated liver fibrosis	Diet-induced NASH mice experiments
[102]	Administration of human α-defensin-5 in mice under obesogenic conditions improved liver lipid profile and prevents liver steatosis	High-fat diet mice experiments
[112]	Lactoferrin administration reduced liver lipogenesis and inflammation and improved hepatic steatosis	High-fat diet mice experiments
[113]	Lactoferrin administration improved hepatic lipid metabolism, liver function and hepatocellular iron homeostasis, and inhibits endoplasmic reticulum stress and inflammation	Experiments in genetic obesity (ob/ob) mice model
[114]	Lactoferrin administration prevented hepatic injury, inflammation, and fibrosis in NASH via NF-κB inactivation	Experiments in NASH rat model

endotoxin-mediated inflammatory pathway.[111] Similar protective effects of lactoferrin in the prevention of liver steatosis by decreasing lipogenic and inflammatory mediators in mice fed with high-fat diet,[112,116] mice with genetic obesity,[113] and rats fed with high-fat diet and intraperitoneally injected with dimethylnitrosamine[114] were also reported. Consistent with these studies, a recent work confirmed that lactoferrin supplements and specific probiotic strains separately exert a role in the control of MASLD, and demonstrated that probiotics expressing recombinant lactoferrin showed synergistic effects enhancing their efficacy in improving hepatic steatosis.[115]

6 | THE 'BUFFERING EFFICIENCY HYPOTHESIS' IN THE CONTEXT OF MASLD

According to the 'buffering efficiency' hypothesis,[73] obesogenic lifestyle and obesity-associated metabolic disturbances disrupt the buffering efficiency of body defence barriers, increasing intestinal permeability and pro-inflammatory antimicrobial proteins, but reducing those antimicrobial proteins that buffer microbial products. The recovery of innate immunity buffering efficiency though promoting endogenous biosynthesis

or exogenous administration of immunomodulatory antimicrobial proteins (such as lactoferrin or defensins) might be a plausible therapeutic approach to prevent and treat MASLD.

7 | CONCLUSION

There is a large number of experimental and clinical studies supporting the hypothesis that changes of intestinal microbiota composition and impairments of intestinal barrier function, including the loss of tight junctions but also changes in antimicrobial peptides, all adding to an increased permeation of bacterial endotoxin and other PAMPs and the induction of TLRs signalling cascades in the liver, are critical in the development of MASLD. The most relevant studies showing the importance of intestinal barrier dysfunction, endotoxin, LBP, lipocalin 2, defensins and lactoferrin on MASLD were summarized in Table 1. Studies further suggest that these alterations are related to the prevalence of overweight and obesity, but also specific dietary patterns including diets rich in saturated fats and sugars like fructose. Indeed, while there are several drugs close to being approved for the treatment of MASLD, changes in lifestyle focusing on a loss of body weight but also dietary pattern as well as moderate exercise[117] are still the first line of therapy. And while it has been shown repeatedly that life-style modifications in settings of MASLD are often beneficial not only on the liver but also alterations like intestinal barrier dysfunction and endotoxemia, underlying mechanisms are not yet well understood. Further studies are needed to unravelling the complex interplay of body weight, nutrition, and intestinal microbiota as well as antimicrobial peptides, and intestinal barrier in the development and therapy of MASLD to provide better recommendation not only for the treatment, but even more so the development of this liver disease.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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